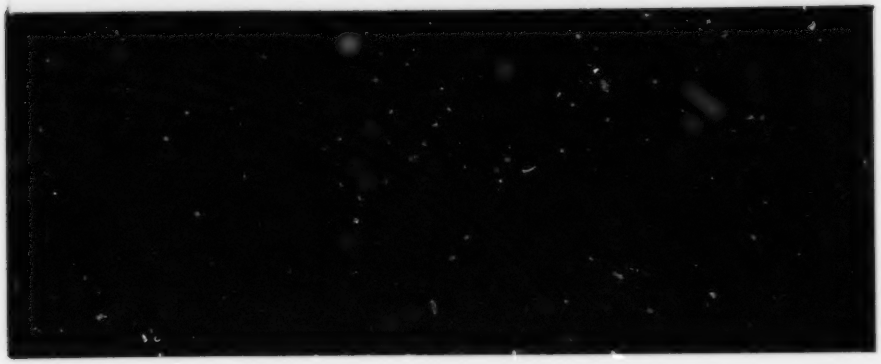
Saskatchewan
Agriculture

Agriculture Development Fund



91-02561/

BLOOD TESTING IN SASKATCHEWAN POULTRY

V-86-06-0071

FINAL REPORT

7th December/88

C. Riddell
Western College of Veterinary Medicine

EXECUTIVE SUMMARY

BLOOD TESTING IN SASKATCHEWAN POULTRY

V-86-06-0071

Abstract

The enzyme linked immunoabsorbent assay was used to measure the effectiveness of vaccination programs against infectious bursal disease, avian encephalomyelitis, infectious bronchitis and Newcastle disease in broiler breeder and leghorn flocks. Inadequate titers for protection were found in several flocks. The test was also used to measure the prevalence and significance of infectious bursal disease and reovirus infection in broiler flocks. The prevalence of reovirus infection was 50% but no correlation was found between infection and flock performance. The prevalence of infectious bursal disease was 43% and a weak, negative correlation was found between infection and body weight at processing.

Summary

In the past decade there has been rapid development of techniques for determining from blood samples whether or not an animal has been infected with a specific disease and the level of protection produced by specific vaccination programs. The enzyme linked immunoabsorbent assay or ELISA test has been developed commercially to rapidly test large numbers of blood samples from poultry. The current project was designed to demonstrate the use of this new technology to various segments of the Saskatchewan poultry industry, to better define the incidence and significance of specific poultry diseases and to study the effectiveness of vaccination programs in Saskatchewan.

Broiler breeder flocks were tested to monitor the effectiveness of vaccinations against avian encephalomyelitis and infectious bursal disease and the protection against infectious bursal disease passed from the breeder hen to the progeny broiler chick. Potential problems were identified in the vaccination programs and recommendations were made to correct them. The hatcheries who obtain hatching eggs from the broiler breeder flocks have been advised to continue monitoring programs for broiler breeder flocks.

Broiler flocks were tested to establish the prevalence and significance of infectious bursal disease and reovirus infection in Saskatchewan. The prevalence of infection was less than 50% for both diseases. No evidence was found of reovirus infection resulting in poor performance. It was recommended that vaccination against reovirus infection is not necessary. Only a few flocks infected with infectious bursal disease performed poorly. Widespread vaccination against infectious bursal disease does not appear necessary. Vaccination on specific problem farms should be considered. Infectious bursal disease damages a small organ found internally above the rectum in the chicken called the bursa of Fabricius. A strong correlation between evidence of infectious bursal disease infection and size of the bursa of Fabricius at processing was noted during the study. Measuring the size of the bursa of Fabricius at processing has been introduced as a simple method for monitoring the presence of infectious bursal disease in broiler flocks. A study of blood samples taken at processing from broiler flocks with production problems identified infectious bronchitis as a significant cause of increased mortalitiy and condemnations. The possibility of vaccinating broiler flocks against this disease is being considered.

Leghorn flocks were tested to measure the effectiveness of current vaccination programs against infectious bursal disease, avian encephalomyelitis, infectious bronchitis and Newcastle disease. Inadequate protection after vaccination against some of these diseases in particular that against infectious bronchitis was discovered. A study of flocks with production problems indicated that infectious bronchitis may be a significant cause of egg production drops and poor egg shell quality. Vaccination programs have been reviewed and possible reasons for poor protection in vaccinated flocks discussed with farmers.

TECHNICAL REPORT

BLOOD TESTING IN SASKATCHEWAN POULTRY

V-86-06-0071

Abstract

10,510 enzyme linked immunoabsorbent assay (ELISA) tests were conducted on blood samples from 30 broiler breeder flocks, 131 broiler flocks and 17 leghorn flocks.

Broiler breeder flocks were tested to measure the effectiveness of vaccination against avian encephalomyelitis and infectious bursal disease. There was inadequate vaccination for infectious bursal disease in 3 of 13 flocks and for avian encephalomyelitis in 9 of 16 flocks. Revaccination of 2 of the 3 flocks inadequately vaccinated against infectious bursal disease resulted in satisfactory titers. Progeny chicks from the broiler breeder flocks were tested to measure the protection against infectious bursal disease obtained from the vaccinated hen. Progeny from 3 of 16 flocks had inadequate protection. Recommendations were made to the hatcheries who supervise broiler breeder flocks to modify vaccination protocols and to continue monitoring.

Broiler flocks were tested at processing to establish the significance and prevalence of infectious bursal disease and reovirus infection on Saskatchewan farms. The prevalence of infectious bursal disease and reovirus infection were 43% and 50%, respectively. No correlation was found between reovirus infection and body weight and condemnations at processing. A weak negative correlation was found between infectious bursal disease infection and body weight at processing. Widespread vaccination of broiler flocks against infectious bursal disease does not appear necessary but may be indicated on specific farms. A strong correlation was found between infectious bursal disease and size of the bursae of Fabricius at processing. Weighing of bursae at processing has been introduced as a simple inexpensive method of monitoring for infectious bursal disease. Fifteen broiler flocks with production problems were tested for evidence of infection with infectious bursal disease, reovirus, Newcastle disease and infectious bronchitis. Four of the 15 flocks had been exposed to infectious bronchitis and had high condemnations for airsacculitis.

Leghorn flocks were tested at 20, 30, 40, 50 and 60 weeks of age to measure the effectiveness of vaccination against infectious bronchitis, Newcastle disease, avian encephalomyelitis and infectious bursal disease. Titers were satisfactory against avian encephalomyelitis and infectious bursal disease but in 5 flocks titers against infectious bronchitis were unsatisfactory. Five leghorn flocks with production problems were tested for titers against infectious bronchitis. Three of these flocks had evidence of field exposure to infectious bronchitis. Vaccination programs have been reviewed and possible reasons for poor results discussed with farmers.

Background and Objectives

In the past decade there has been rapid development of techniques for measuring the level of antibodies against common infectious agents in domestic poultry. In particular, a specific test, the enzyme linked immunoabsorbent assay (ELISA) has been developed to test rapidly large numbers of blood samples from poultry flocks. Commercial companies have developed kits for the ELISA test and have also developed software to allow rapid handling, storage and graphical presentation of results. The ELISA test offers great potential for defining the exposure of a flock to specific infectious agents, the possible effect of subclinical disease on flock performance, and the effectiveness of vaccination programs. Many integrated poultry companies in the United States of America have started to use this technology. The purpose of the present study was to demonstrate the use of this new technology to the Saskatchewan poultry industry, to use the technology to better define the incidence and significance of specific poultry diseases and effectiveness of vaccination programs in Saskatchewan and to use this data to improve disease control in poultry in Saskatchewan.

Experimental Methods

The significance of various infectious diseases and the vaccination programs utilized to control these diseases vary between different segments of the Saskatchewan poultry industry. As a result, different protocols were designed for broiler breeder flocks, broiler flocks and leghorn flocks and each of these segments of the industry will be discussed separately.

Broiler Breeder Flocks. The level of antibody resulting from vaccination programs in the broiler breeder chicken is important for the health of the broiler breeder flock but is also important in the progeny broiler flocks. Antibody is transmitted from the breeder hen through the egg to the chick. Such maternal antibody is extremely important in protecting the baby chick from early challenge with infectious disease on the broiler farm. In a prior preliminary study conducted with the cooperation of the University of Georgia, blood samples were collected from day-old cull chicks from the majority of broiler breeder flocks in Saskatchewan. At each sampling blood was taken from 20 day-old cull chicks and the ELISA test used to measure the levels of antibody against infectious bursal disease virus, reovirus, infectious bronchitis virus and Newcastle disease virus in these chicks. Many chicks had inadequate antibody levels; in particular against infectious bursal disease. These poor antibody levels were attributed to inadequate early vaccination of breeder hens during the rearing period. The vaccination program was revised and blood sampling of cull chicks and ELISA testing for infectious bursal disease antibody levels continued in the present study. In addition 20 blood samples were collected from the broiler breeder flocks at 7 weeks and 16 weeks of age to check whether or not early vaccinations against infectious bursal disease and avian encephalomyelitis had produced satisfactory results. The hatcheries who supervise the broiler breeder flocks were encouraged to collect blood samples for many of these tests with the help and advice of the poultry extension personnel at the University of Saskatchewan.

Broiler Flocks. Reovirus and infectious bursal disease virus have been demonstrated experimentally to cause significant disease problems in broiler

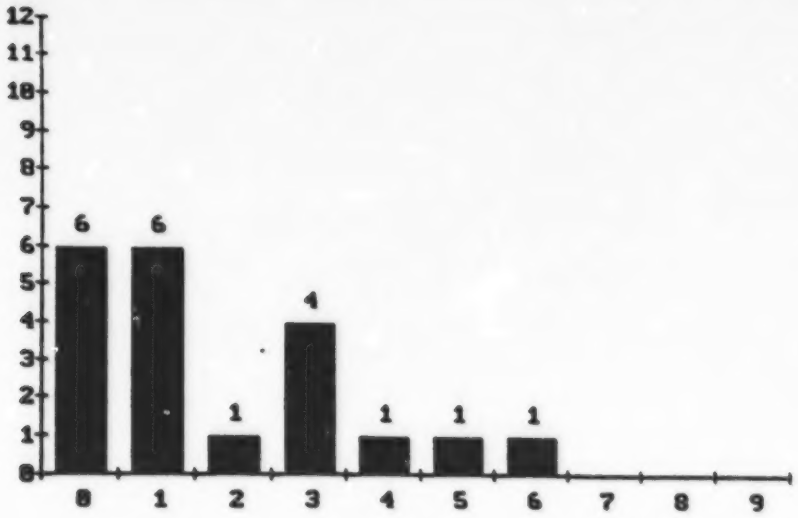
chickens. Clinical disease due to infectious bursal disease but not due to reovirus infection has been recognized in Saskatchewan broiler chickens. The possible significance of these infectious agents as causes of subclinical disease in Saskatchewan broiler chickens is unknown. 20 blood samples were collected at processing from 2 different broiler chicken flocks from every broiler chicken farm in Saskatchewan. These samples were tested for antibodies against infectious bursal disease virus and reovirus. Bursae of Fabricius, the organ affected by infectious bursal disease, were collected and weighed from the same flocks at one processing plant. The weights of bursae and body weights adjusted to a 50 day weight, of the birds at processing, adjusted to a 50 day weight, were compared with antibody titres in an attempt to determine whether there was any correlation between decreased weights and infection. In addition to this survey of all broiler farms in Saskatchewan blood samples were collected at processing from flocks with production problems. These samples were tested for antibodies against infectious bursal disease virus, reovirus, infectious bronchitis virus, and Newcastle disease virus.

Leghorn flocks. Egg production drops and poor egg quality have caused significant problems on Saskatchewan farms. In some cases these problems have been attributed to infectious diseases against which many farmers vaccinate their chickens. To attempt to evaluate current vaccination programs, 12 leghorn flocks scattered throughout the province were asked to cooperate in the study. 20 blood samples were collected from these flocks at 20,30,40,50 and 60 weeks of age. The 20 week samples were tested for antibodies against infectious bronchitis virus, Newcastle disease virus, avian encephalomyelitis virus and infectious bursal disease virus using the ELISA test. The subsequent samples were only

tested for antibodies against infectious bronchitis and Newcastle disease. For each flock tested a detailed history on the vaccination program utilized was obtained. The cooperating producers agreed to support the program by paying for the initial 20 week test.

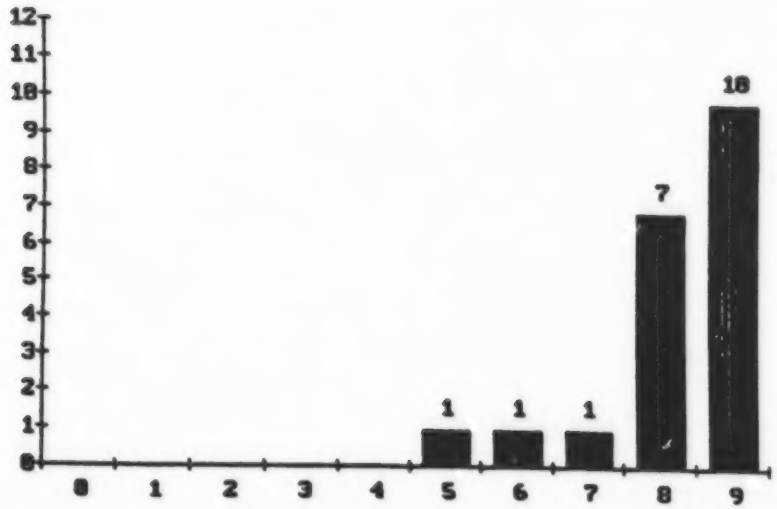
Processing of blood samples

Sera were separated from all blood samples. The sera were held frozen prior to testing. All ELISA tests were run on the Flockchek system (Agritech Systems, Ind. 100 Fore Street, Portland, Maine 04101). Flockchek results are reported numerically and graphically (Fig. 1). Individual titers are grouped into 9 profile groups from 0 - 9 which represent increasing levels of antibody. Profile group of titer which is considered protective varies for different diseases but a rough guide is that the minimum titer for protection is at least group 3-4. Much higher titers are desirable in specific situations. The titers for each flock are also reported as a mean titer. A coefficient of variation is reported as a measure of variability between individual titers for each flock. The coefficient of variation is the standard deviation expressed as a percentage of the mean titer. In evaluating effectiveness and uniformity of vaccination programs a rough guide is that the coefficient of variation should be 30 percent or less. In general high titers are associated with low coefficients of variation and low titers are associated with high coefficients of variation.



ASSAY : BRONCHITIS STD. DEV. : 1515.13

MEAN TITER : 1598 COEFF. VAR.% : 94.83



ASSAY: BRONCHITIS STD. DEV. : 3085.09

MEAN TITER : 9731 COEFF. VAR.% : 31.70

Fig. 1 Results of ELISA testing of 20 bloods from two different flocks for levels of antibody against infectious bronchitis at 20 weeks of age. Top. Flock was given 3 live vaccines against infectious bronchitis during the growing period. Titers are poor and many birds may not be protected. Bottom. Flock was given 2 live vaccines and 1 killed vaccine against infectious bronchitis during the growing period. Titers are excellent.

Results and Discussion

Broiler breeder flocks.

1) Antibodies against infectious bursal disease at 7 weeks of age.

Of 13 flocks studied 10 flocks had mean titers ranging from 3028 to 6619. These 10 flocks were considered to have been adequately vaccinated. 3 flocks had average titres of 648, 1332 and 1386 which were considered inadequate. In particular a titer of 648 suggested that vaccination may not have been done or done incorrectly. Revaccination of these flocks produced satisfactory titers at a later age in 2 of the flocks from which later blood samples were obtained.

2) Antibodies against avian encephalomyelitis at 15 weeks of age.

Of 16 flocks studied 7 flocks had average titers ranging from 2143 - 3873. These 7 flocks were considered to have been adequately vaccinated. Titers were not as high as with infectious bursal disease. Such a difference between two different diseases is not unexpected. Nine flocks had average titers of 751 - 1884 which were considered inadequate. In particular a titer of 751 suggested that vaccination may not have been done in one flock. No disease problems related to avian encephalomyelitis have been encountered to date in any of these flocks. Further study is needed to determine why some flocks had such low titers.

3) Antibodies against infectious bursal disease in chicks derived from the breeder flocks.

Of 16 flocks studied 13 flocks had average titers ranging from 4882 - 7271. These flocks were considered to have been adequately vaccinated. Three flocks had average titers of 3101, 3882 and 4262 which were considered inadequate. Bloods were not collected from these later flocks at 7 weeks of age. Previous studies have suggested that similar low titers were due to inadequate initial vaccinations. The hatcheries which supervise the vaccination programs for the breeder flocks have been advised that titers have been inadequate for both infectious bursal disease and avian encephalomyelitis. They have been advised to continue the monitoring program initiated by the poultry extension service and supported in part by this study.

Broilers.

a) Antibodies against infectious bursal disease virus and reovirus at processing.

Considerable variation in titers was noted in the 116 flocks studied. Based on lack of any evidence of damage to the bursa of Fabricius, 66 flocks with titers from 17 to 1158 against infectious bursal disease had probably never been exposed to infectious bursal disease while the remaining 50 flocks which had been exposed had titers varying from 1381 to 7865. 58 flocks with titers from 246 to 1152 against reovirus probably never had been exposed to reovirus infection, while 58 flocks which had been exposed had titers varying from 1240 to 4808.

b) Correlation between antibody titers and performance of flocks.

A summary of correlation coefficients and their significance is presented in Table I. There was a strong negative correlation between antibody titers against infectious bursal disease virus and bursa/body weight ratio. This correlation can be explained by the destruction of the bursa which occurs during

Table 1. Correlation between titers against infectious bursal disease virus (IBDV) and reovirus (RV) and 50 day body weight, bursa/body weight ratio and incidence of condemnations.

	50 day body weight	Bursa/body weight ratio	Incidence of condemnations
IBDV titer	-0.18[A] (0.05[B])	-0.85 (0.0001)	-0.04 (0.67)
RV titer	-0.06 (0.49)	-0.10 (0.50)	-0.15 (0.11)

[A] Correlation coefficient
[B] Significance

infection with infectious bursal disease virus. A reduction in size of the bursa will occur in other diseases but these results suggest that small bursae in broiler chicken flocks at processing in Saskatchewan are probably due to infectious bursal disease. Weighing bursae from a flock at processing is a simple monitoring procedure which should give a measure of exposure to infectious bursal disease and severity of resulting bursal damage in that flock. This simple monitoring procedure has been started by two processing plants in Saskatchewan. The only other significant correlation was a weak negative

correlation between antibody titers against infectious bursal disease and 50 day body weight. This was interpreted to indicate that a flock infected with infectious bursal disease would probably have poorer performance but that not all poor performance could be attributed to infectious bursal disease. There are two possible methods of control of infectious bursal disease, in broiler chickens, eradication or vaccination. The large numbers of flocks free of infectious bursal disease virus has led the Poultry Extension service to recommend the former procedure. Vaccination may be necessary on certain farms where eradication appears impossible. The study provided evidence of reovirus infection in many flocks but no evidence that it resulted in decreased production. The poultry extension service is recommending against the use of reovirus vaccines. There was no evidence that either infectious bursal disease or reovirus infection affected the incidence of condemnations.

c) Flocks with production problems.

In ELISA testing at processing of 15 flocks with varied production problems including poor growth rate, increased mortality and high condemnations 4 flocks had titers against infectious bursal disease, 5 flocks had titers against reovirus and 4 flocks had titers against infectious bronchitis infection at processing. The results confirmed that neither infectious bursal disease nor reovirus infection are consistently present when there is poor production. Widespread vaccination against these diseases may be unnecessary. Infectious bronchitis disease appears to be restricted to a few farms. Titers against infectious bronchitis coincided with high condemnations due to airsacculitis. To date this infection has not been regarded as a significant problem in

Saskatchewan but results of this study suggest that it may be more widespread and may be causing more damage than had been realized previously. Consideration is being given to starting vaccination of broiler flocks against this disease.

Leghorn Flocks

1) Antibodies against avian encephalomyelitis at 20 weeks of age.

Only 4 out of 12 flocks studied were vaccinated against avian encephalomyelitis, a disease which if it occurs during egg production can cause significant drops in egg production. These 4 flocks had adequate levels of antibody (mean titers 4346 - 5700) against avian encephalomyelitis at 20 weeks of age indicating that they had been satisfactorily vaccinated or had been infected by a field virus during the growing period. Three unvaccinated flocks also had antibodies (mean titers 1458 - 2887) against avian encephalomyelitis indicating that they had been naturally infected probably by a field virus. The remaining 5 flocks had no antibodies (mean titers < 700) against avian encephalomyelitis indicating that they were susceptible to infection. The data indicates that there is a risk of avian encephalomyelitis infection in Saskatchewan and that more widespread vaccination against this disease would be desirable. However on multiple age farms there is a risk to vaccination as the vaccine virus is virulent and can spread from a vaccinated flock of young growing birds to a susceptible laying flock on the farm and cause clinical disease.

2) Antibodies against infectious bursal disease at 20 weeks of age.

10 out of 12 flocks were vaccinated against infectious bursal disease. These flocks had adequate levels of antibody (mean titers 2801 - 7357) against infectious bursal disease at 20 weeks of age indicating that they had been satisfactorily vaccinated or had been infected by a field virus during the growing period. Two flocks were unvaccinated and had no antibodies (mean titers < 800) against infectious bursal disease. Infectious bursal disease is considered less contagious than avian encephalomyelitis and if proper isolation and sanitation programs are maintained vaccination programs may not be needed. However current vaccination programs appear to be performing adequately in Saskatchewan.

3) Antibodies against infectious bronchitis.

All flocks studied except one were vaccinated against infectious bronchitis but there was variation in products used, routes of administration and programs. Considerable variation in mean titers was noted and also considerable variation in titers between individual birds within a flock.

The poorest result at 20 weeks was an average titer of 1598 and the best result at 20 weeks was an average titer of 9731. The coefficient of variation for the former was 95 while the coefficient of variation for the latter was 32. Figure 1 illustrates the difference in titers between these flocks. In following individual flocks throughout their production year there was considerable variation in some flocks in average titers from one age to another. An example of such variation is shown in Table 2. Statistical analysis using analysis of variance indicated that the differences from one age to another were significant.

Table 2. ELISA titers against infectious bronchitis virus through the production life of a flock.

Weeks of age	Mean Titer	Coefficient of Variation
20	3619	54
30	2503	71
40	5196	57
50	2175	66
60	5091	49

In 5 flocks the titers were considered inadequate and may not protect the flocks against challenge by a field virus. No obvious egg production problems were reported in the flocks studied. In reviewing vaccination programs with farmers possible reasons for poor antibody levels discovered included improper handling and administration of the vaccine. The results raised questions about the effectiveness of giving live vaccines in the drinking water as opposed to giving the same live vaccines by aerosol spray. The best results were obtained by a program in which birds were given two live vaccinations in the drinking water at 3 and 9 weeks of age followed by a killed vaccine given to each bird by injection at 16 weeks of age. Most producers are reluctant to use such a program because of the extra labour needed to handle each bird and because killed vaccines are more expensive than live vaccines. The variation in titers through the production cycle of some flocks would suggest that the flocks are being challenged with field viruses and this is boosting immunity. It is possible that this could result in production problems and further study is needed.

4) Antibodies against Newcastle Disease.

Results were very similar to those reported for infectious bronchitis.

5) Flocks with production problems.

Blood samples were collected from 5 flocks with production problems. Three of these flocks had titers 7612, 8136 and 11,343 against infectious bronchitis. These titers were higher than titers obtained by other flocks which used similar vaccination programs and suggested field exposure. It was considered probable that the production problems were due to infectious bronchitis and that vaccination programs had been inadequate.

Conclusions and Impact.

The study demonstrated the usefulness of the ELISA test in determining the incidence and significance of specific diseases and the effectiveness of vaccination programs in the Saskatchewan Poultry Industry. Some segments of the industry have started to use the test as a regular monitoring procedure. The study has helped the poultry extension service funded by the poultry industry and based at the University of Saskatchewan advise on disease control programs. It has also provided for the extension service a useful data bank covering the incidence of and antibody titers against important infectious diseases in the Saskatchewan poultry industry.

Talks given

Date	Meeting	Title
March 4/88	Saskatchewan Poultry Industry Conference, Saskatoon	ELISA testing and flock profiles
November 8/88	Saskatchewan Egg Producers' Marketing Board, Swift Current	Blood testing study of effectiveness of vaccination programs
November 9/88	Saskatchewan Egg Producers' Marketing Board, Saskatoon	Blood testing study of effectiveness of vaccination programs
November 9/88	Saskatchewan Chicken Producers' Marketing Board, Dalmeny	Blood testing at processing in broilers
November 15/88	Saskatchewan Chicken Producers' Marketing Board, Wynyard	Blood testing at processing in broilers

List of Personnel

	Position	Period	Percentage of Time
C. Riddell	Professor	Feb. 87 - Nov. 88	10
K. Little	Extension Poultry Veterinarian	Feb. 87 - May 88	10
F. Tietge	Technician	Feb. 87 - May 88	50
R. Springer	Technician	June 88 - August 88	50